

Session : The Cannabis Plant: A Sustainable Source of Bioactive Molecules and New Pre- and Post-Biotics of Nutritional and Medical Importance

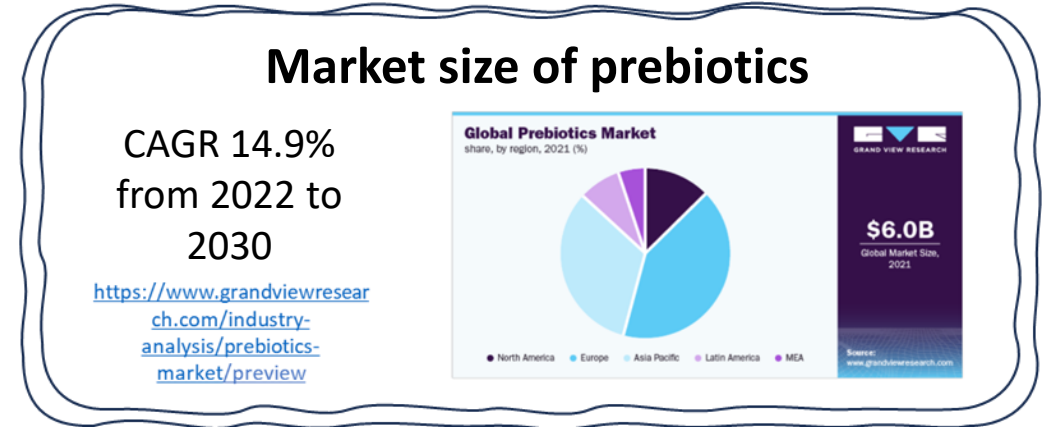
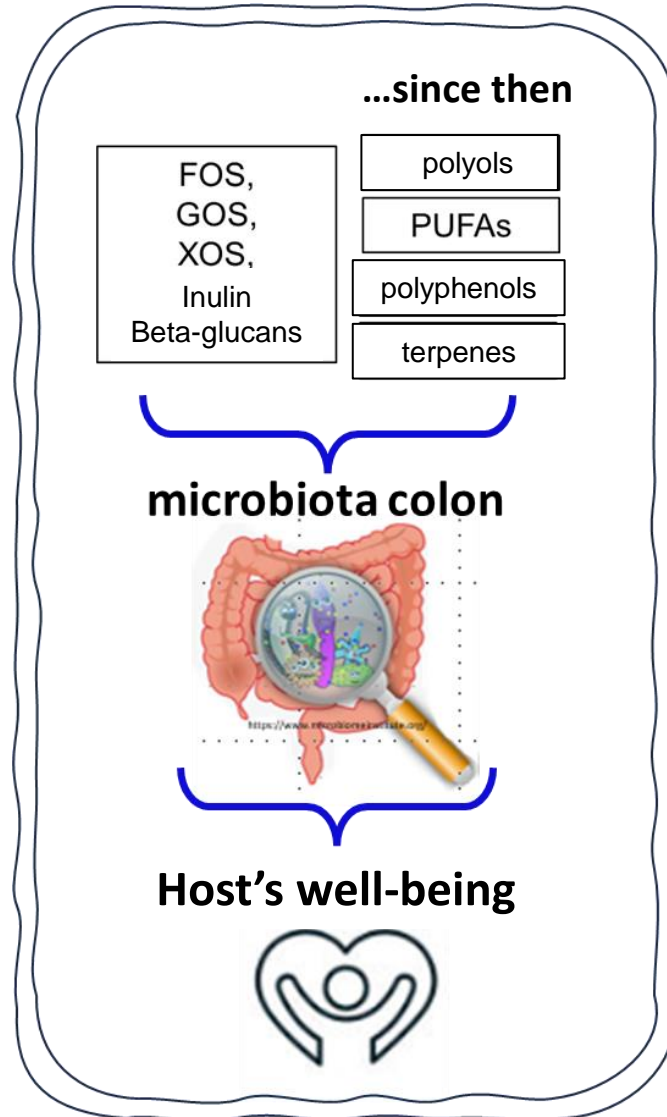
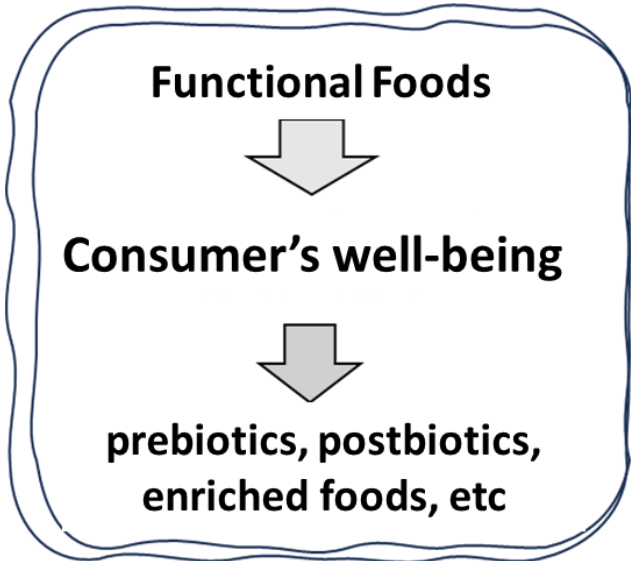
Functionalities of Cannabis Fiber on Gut Microbiota Fermentation: an hemp postbiotic?

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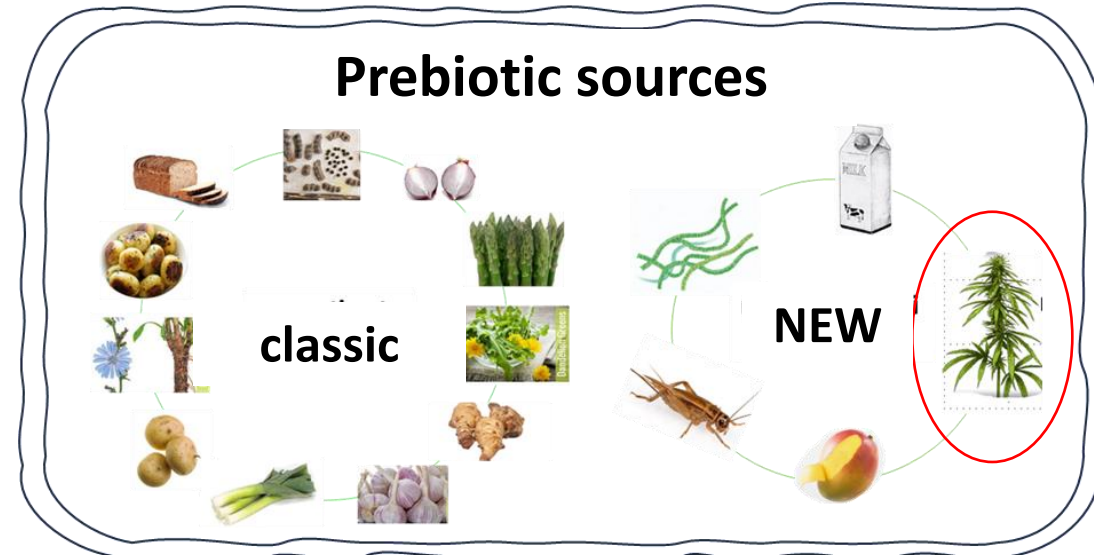
Prebiotics, Probiotics, Postbiotics = Functional foods



ISAPP CONSENSUS STATEMENT


2017 **Prebiotic:**
a substrate that is selectively used by microorganisms and confers a health benefit on the host

2022 **Postbiotic:**
Preparation of inanimate microorganisms and/or their components that confers a health benefit on the host



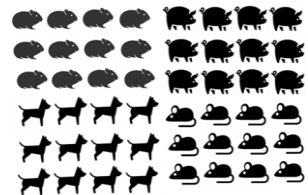
The role of *in vitro* gut models

To study the effect of foods on the gut microbiota, there are three approaches, which can also be complementary or substitutes

1)  **Clinical trials**

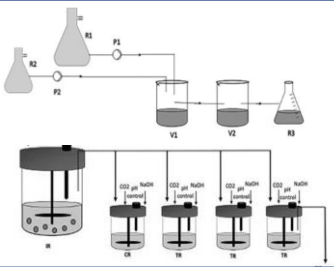
- Gold standard
- Variability

- Expansive
- Time cost
- Healthy subjects

2)  **Animal models**

- Host Interaction
 - *in vivo*
- Variability




- Ethical issues
- Growing stage
- Traslatability

3)  ***in vitro* models**

- Short time
- Traslatability
- Pre-clinical

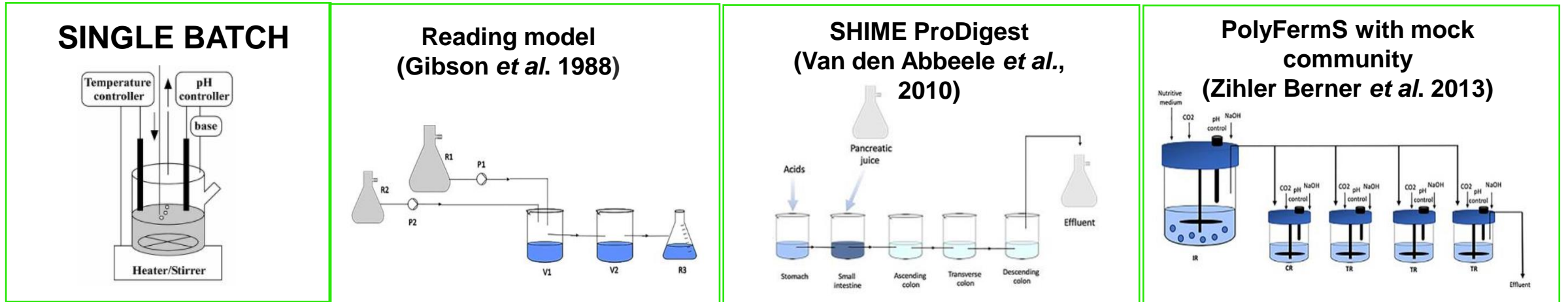
- Host interaction
- Variability
- Simulation

The 3 R's of Animal Research

<p>Reduce</p>  <p>Reduce the number of animals used</p>	<p>Refine</p>  <p>Refine tests to cause animals less stress</p>	<p>Replace</p>  <p>Replace animal studies with other methods</p>
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The evolution of *in vitro* gut models to study functionalities of foods

different models for different applications



Standardization/
reproducibility



complexity

«SHORT TERM» days

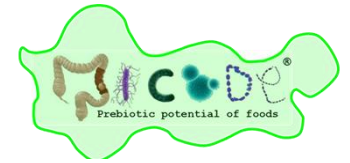
in vivo resemblance

«LONG TERM» weeks

- *Pre-clinical Screening*
- *Precision Nutrition*
- *Personalized Nutrition*

Experimental implementation of MICODE

to study the impact of foods or food supplements on gut microbiota



REFORMULATION AND COOKING METHODS

Byproducts enriched bread

Spirulin-enriched bread

NO_x free salami

Frying vs Roasting

Functional GF breads

LACTOSE-FREE PRODUCTS

Milk

Whey

FOOD SUPPLEMENTS

Hempseed bran

Prebiotic supplement

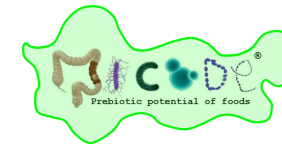
ANTIBIOTICS

Amoxicillin

Published papers 2020-2023

- *Food Bioscience*, 53, 102568, ISSN 2212-4292 <https://doi.org/10.1016/j.fbio.2023.102568>.
- *Food & Function* (14), 3509-3525, <https://doi.org/10.1039/D3FO00085K>
- *Scientific Reports* 13, 1552. <https://doi.org/10.1038/s41598-023-27726-w>.
- *Int. J. of Food Sc. and Tech.* 58(8) 4485-4494 <https://doi.org/10.1111/ijfs.16253>.
- *Appl. Microb. and Biotech.* 106, 7595-7614, <https://doi.org/10.1007/s00253-022-12223-3>
- *Food Res. Int.*, 111702. <https://doi.org/10.1016/j.foodres.2022.111702>
- *Foods* 10(10), 2371 <https://doi.org/10.3390/foods10102371>
- *Food & Function* 12, 10226 - 10238. DOI: [10.1039/D1FO01239H](https://doi.org/10.1039/D1FO01239H)
- *Nutrients*, 27;13(3):787. <https://doi.org/10.3390/foods10102371>
- *Food Chemistry*, 2023 in press
- *Metabolites*, 12, 736. <https://doi.org/10.3390/metabo12080736>
- *FEMS Microbiology Letters*, 367(12), 1-10 fnaa097 <https://doi.org/10.1093/femsle/fnaa097>

MICODE workflow



**FOODS,
PREBIOTICS,
PROBIOTICS**

1



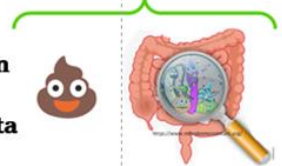
COLONIC FERMENTATION

2



Ethical Committee
3 or more donors
inclusion criteria

prompt extraction
and purification
of colon microbiota

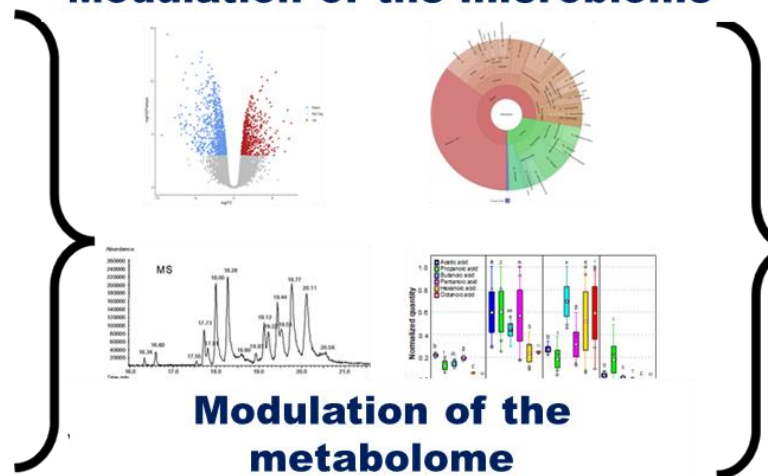


3

qPCR
and/
or
16S NGS



Modulation of the microbiome



4

GC/MS
and/
or
NMR

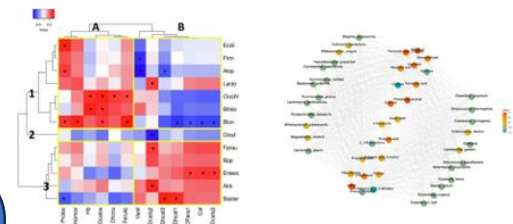


**Modulation of the
metabolome**

Changes over time in
respect to the **BASELINE**
of fermentation

↓

**BASELINE = Microbiota
adaptation to the *in vitro*
conditions**



5

**INTEROMIC
microbiome/metabolome**

OMIC DATA

↓

ECOLOGICAL INDICATORS

↓

**FUNCTIONALITY
on GUT
MICROBIOTA**

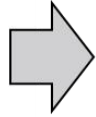
+ QCs

- Cultivation of *Archea*
- Microbiota diversities
- Prebiotic paradigm
- Stool ubiquitous VOCs

focus of this research



**SEEDS
from
Hemp**



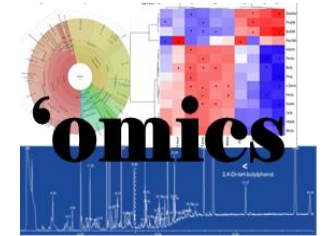
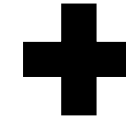
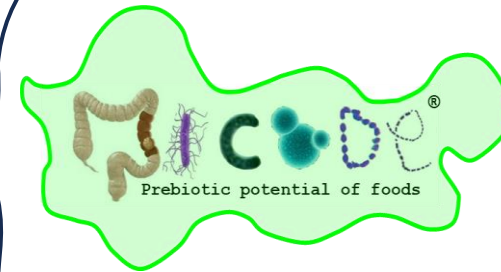
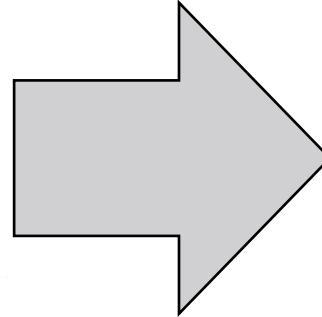
- Gluten free
- Omega3/Omega6 = 2
- Rich in proteins, AAs
- Rich in fibers, minerals
- 5 Billion market size

BY-PRODUCT

20% of wastes from
whole seed
decortication



FOOD SUPPLEMENT



scientific reports

Article | [Open Access](#) | Published: 27 January 2023

Beneficial metabolic transformations and prebiotic potential of hemp bran and its alcalase hydrolysate, after colonic fermentation in a gut model

[Lorenzo Nissen](#), [Flavia Casciano](#), [Elena Babini](#) & [Andrea Gianotti](#)

[Scientific Reports](#) 13, Article number: 1552 (2023) | [Cite this article](#)

Setti, Samaei, Maggiore,
Nissen, Gianotti, Babini 2020
Food Bioproc Technol, 151,112201

Nissen, Casciano,
Babini, Gianotti, 2021
LWT - Food Sci Technol,151,112201

ANTI-OXIDANT

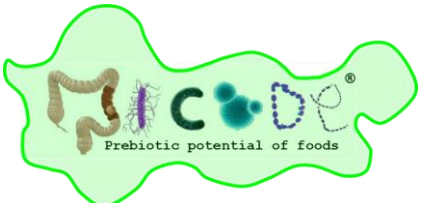
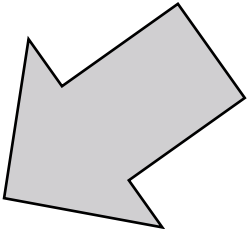
PREBIOTIC

Materials and methods

1)  **HB = Hemp seed bran**

2)  **HBPA = alcalase treated HB**

3) **FOS = Fructoligosaccharides**



Batch short-term DISTAL COLON

3 healthy donors
24 h, 4 time points



HBPA HB FOS blank



MiSeq
qPCR

MICROBIOMICS

- Ecological diversities
- Beneficial taxa
- Opportunistic taxa
- Prebiotic Index

SPMEGC/MS

METABOLOMICS

- S and MCFAs
- Detrimental VOCs
- Volatilome

INTEROMICS

+ QCs

Results*

1) METABOLOMICS (SPME GCMS, ANOVA & Multivariate)

presented as boxplots, PCA & MANOVA

2) MICROBIOMICS (16S MiSeq, qPCR, ANOVA+)

presented as biodiversities, metataxonomy & $\text{Log}_2(\text{F/C})$

3) INTEROMICS (Spearman, Clusters)

presented as two-way heatmap with dendrograms

***Based on**

<https://doi.org/10.1186/1471-2180-11-219>

<https://doi.org/10.3390/microorganisms8060792>

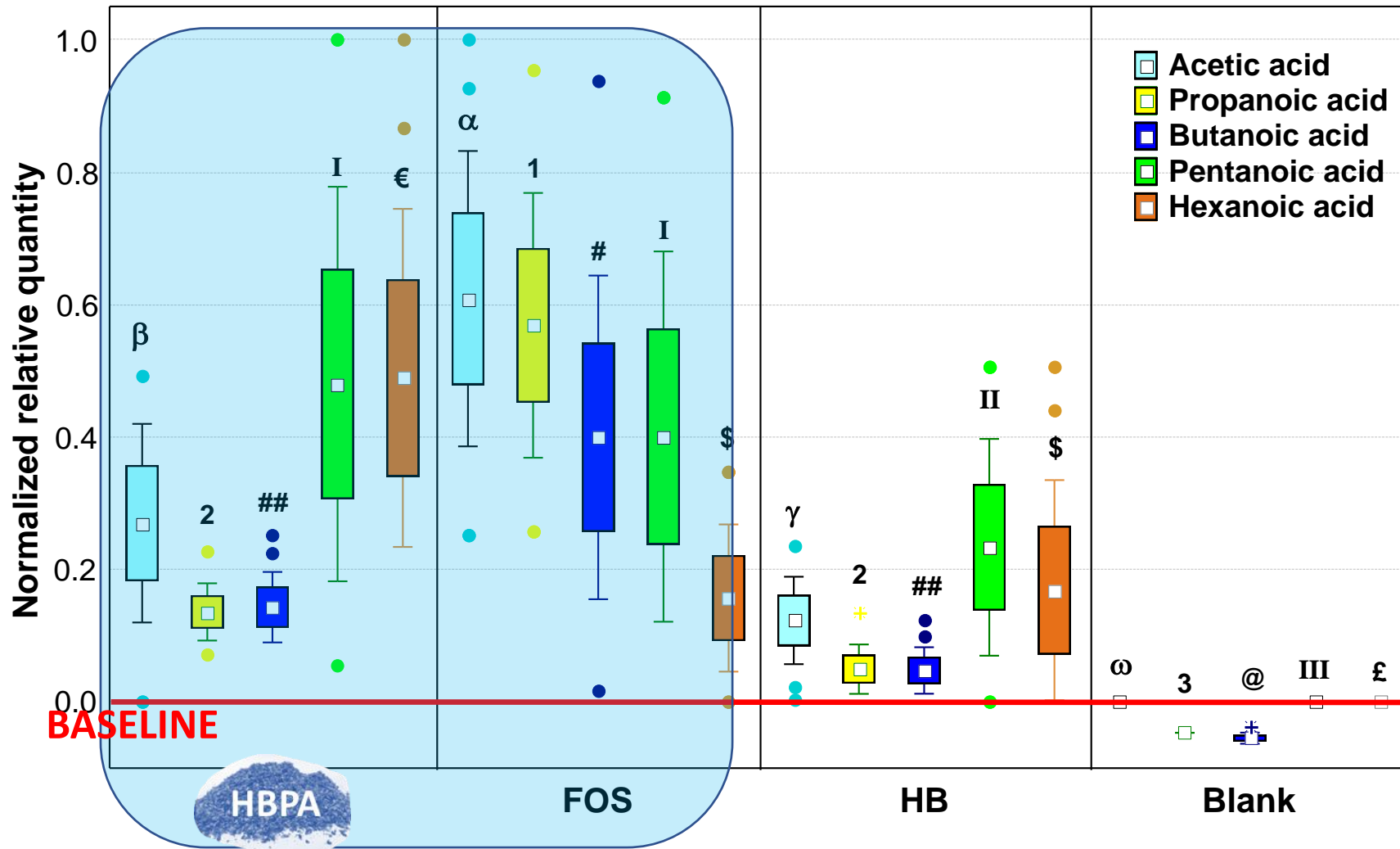
<https://doi.org/10.1016/j.foodres.2020.109029>

<https://doi.org/10.3390/nu9060533>

<https://doi.org/10.1186/s13059-014-0550-8>

<https://doi.org/10.1038/s41598-020-76603-3>

METABOLOMICS: Production of beneficial VOCs



- Mucosal homeostasis
- Energetic metabolism
- Postbiotic substrates
- Anti-inflammatory
 - Anti-oxidants
 - GALT inducers

VOCs	mg/kg ± S.D.	p value [†]
Acetic acid	traces*	0.0010
Propanoic acid	0.012 ± 0.015	0.0467
Butanoic acid	0.101 ± 0.086	0.0424
Pentanoic acid	traces	0.0153
Hexanoic acid	traces	0.0179

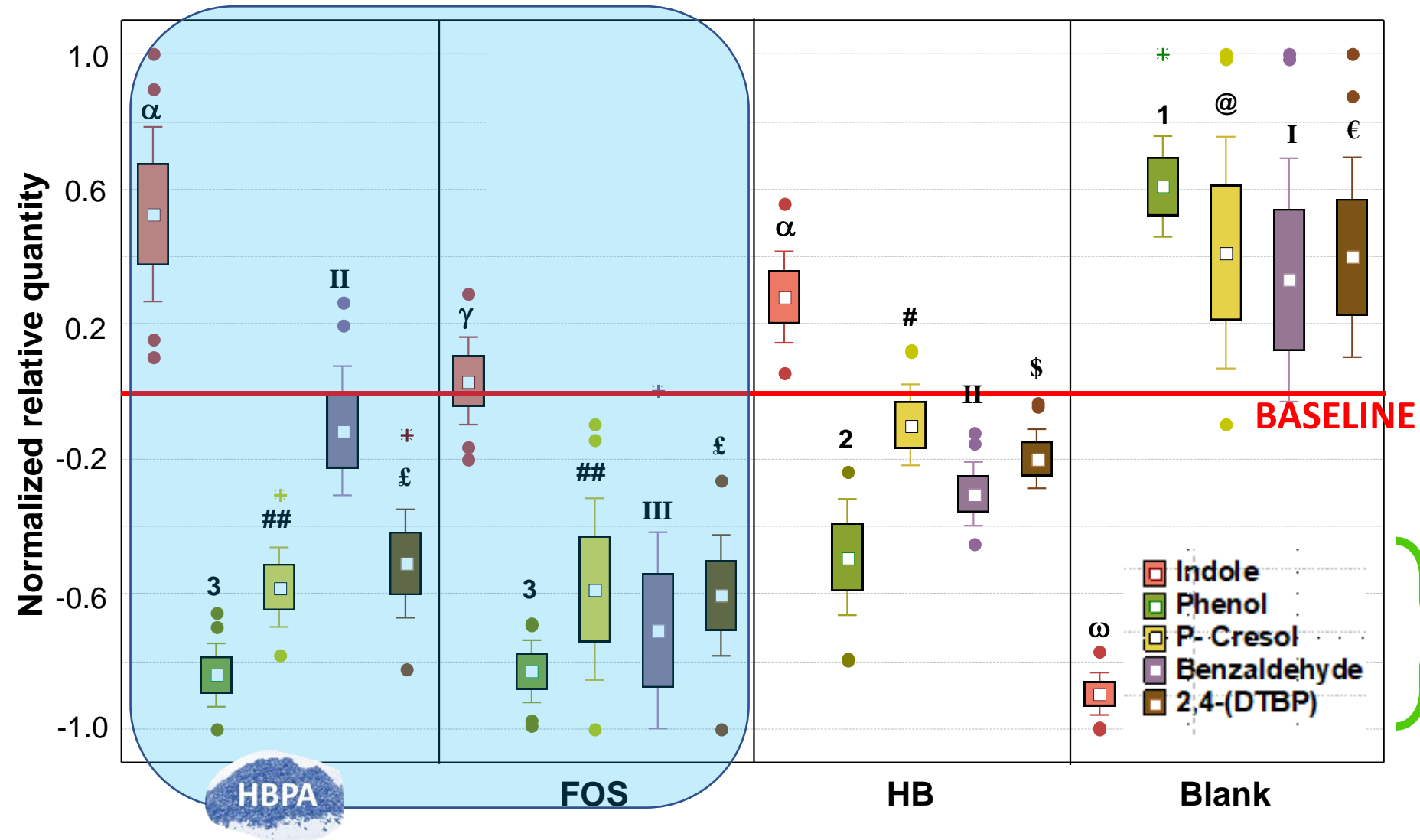
*traces < 0.01 mg/kg; **Phenol, 2,4-bis(1,1-dimethylethyl)-; (LOQ = 0.03 mg/kg and LOD = 0.01 mg/kg); [†]p value of ANOVA from a dataset including all cases and time points.

To a lesser extent than FOS, HPBA is capable of increasing the abundance of the three SCFAs and two MCFAs. Notably, HPBA is capable of producing about twice as much hexanoic acid as FOS

SPME GC/MS

Semi quantified with internal standard 4-methyl. 2-pentanol

METABOLOMICS: Modulation of negative VOCs



VOCs	mg/kg ± S.D.	p value [†]
Indole	7.955 ± 1.388	<0.0001
Phenol	0.177 ± 0.051	<0.0001
Phenol, 4-methyl-	15.022 ± 9.808	0.0141
Benzaldehyde	0.717 ± 0.415	0.0013
2,4-(DTBP)**	1.826 ± 0.624	0.0074

*traces < 0.01 mg/kg; **Phenol, 2,4-bis(1,1-dimethylethyl)-; (LOQ = 0.03 mg/kg and LOD = 0.01 mg/kg); [†]p value of ANOVA from a dataset including all cases and time points.

As FOS, HPBA can modulate the microbiota to produce fewer negative compounds such as Phenol, p-Cresol, Benzaldehyde

- Catabolism trp, tyr, phe
- Mucosal damages
- Dysbiosis inducers
- Pro-inflammatory
 - Lipid-oxidants
- Proteolytic fermentation

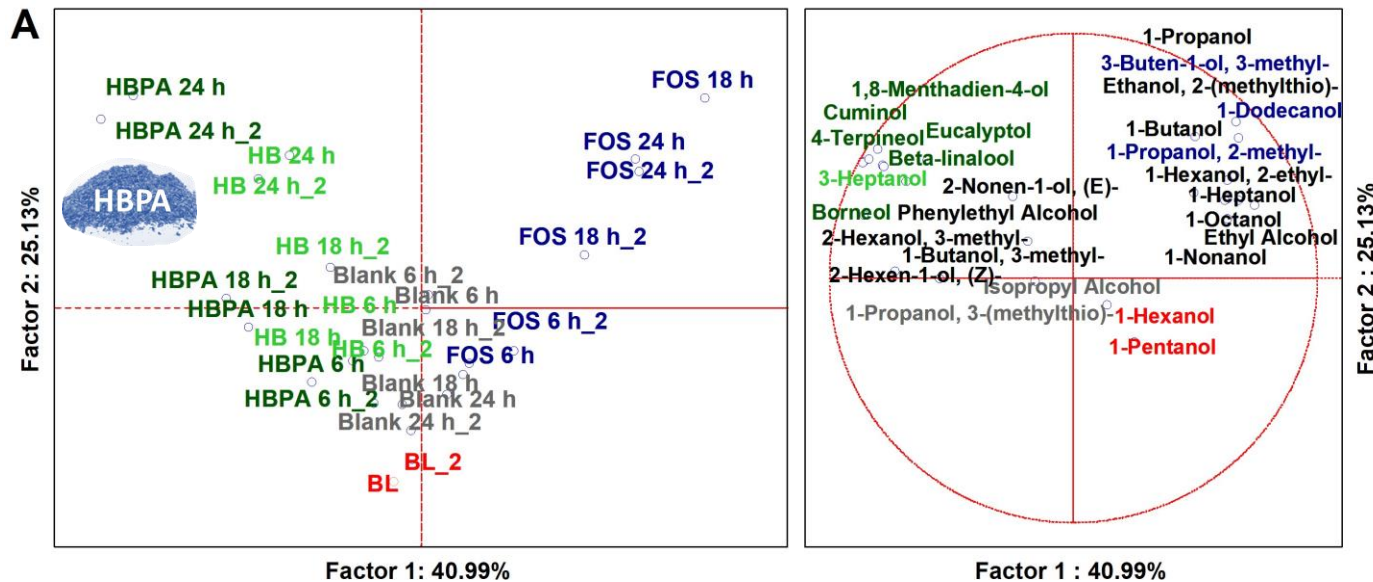
SPME GC/MS

Semi quantified with internal standard benzyl alcohol

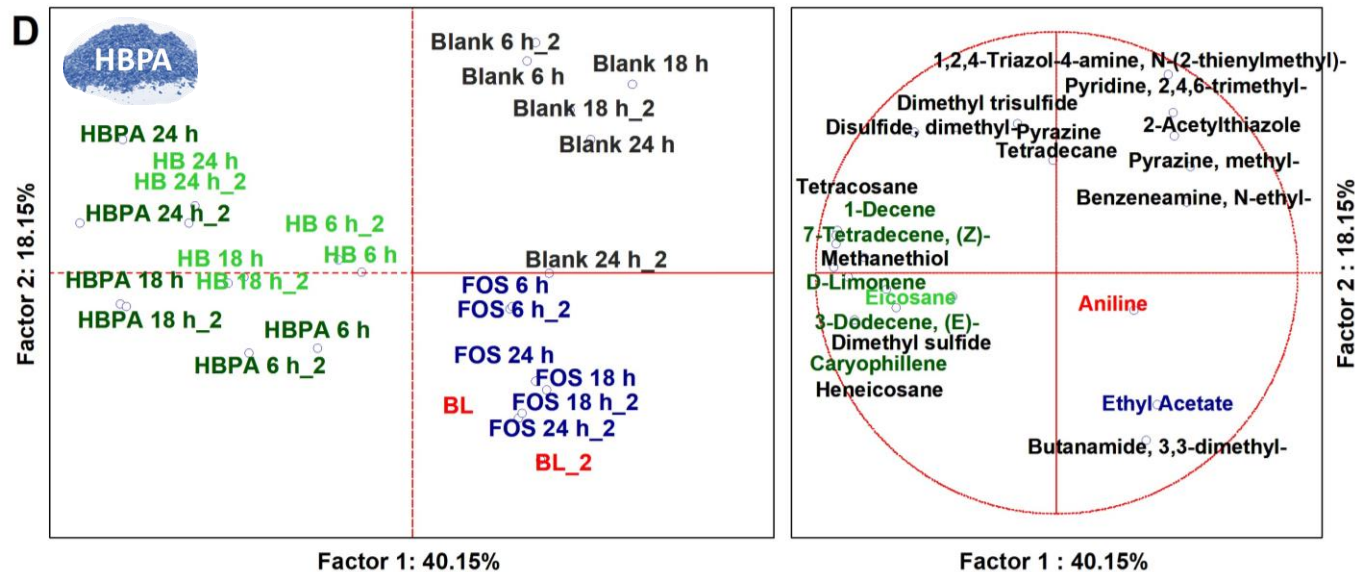
METABOLOMICS: Volatilome reconstruction

SPME GC/MS

Volatilome = VOCs complex of an ecosystem

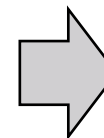


1,8-Menthadien-4-ol
Cuminol
4-Terpineol
Eucalyptol
Borneol



D-Limonene
Caryophyllene
7-Tetradecene (Z)

Discrimination time- and sample-dependent

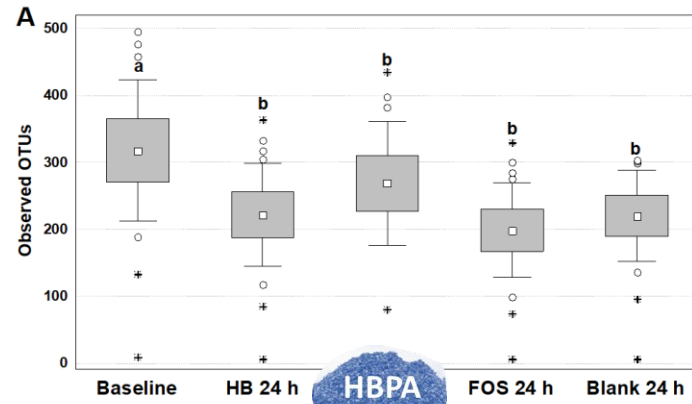


Renown Bioactives

MICROBIOMICS: Biodiversities

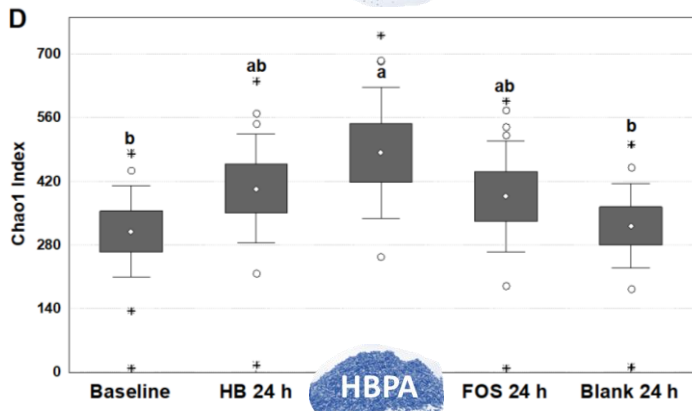
MiSeq

Alpha-diversity



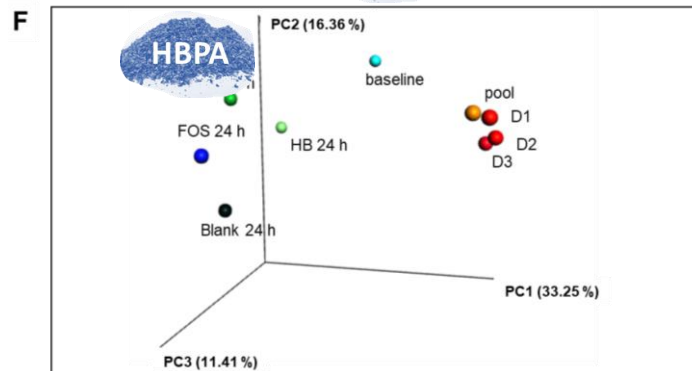
Reduction in microbiota richness after fermentation, but similar compared with FOS

Alpha-diversity



Increased microbiota abundance in respect to the baseline, but similar when compared with FOS

Beta-diversity



HBPA-induced microbial ecology similar to that induced by FOS



Microbiome eubiosis is maintained

MICROBIOMICS: Shifts in the abundance of beneficial taxa

MiSeq

1)

2)

3)

4)

Taxon	% relative abundance	Log ₂ (F/C)				P value
		changes				
	Baseline Mean	HPBA	FOS 24 h	HB 24 h	BC 24 h	
Beneficials and commensals						
<i>Bifidobacterium</i> ;s__adolescentis	4.414 ± 1.743 ^b	↑	↑↑↑	↑	↓	0.03883
<i>Bifidobacterium</i> ;s__bifidum	0.974 ± 0.177 ^b	↑↑	↑↑	↑	↓	0.00132
<i>Bacteroides</i> ;s__acidifaciens	0.115 ± 0.009 ^b	↑	↑	↑	↓	0.00052
<i>Bacteroides</i> ;s__caccae	0.713 ± 0.086 ^b	↑	↑↑	↑	↓↓↓	0.00113
<i>Bacteroides</i> ;s__thetaitaomicron	0.393 ± 0.093 ^c	↑↑↑	↑↑	↑↑↑	↑	0.00001
<i>Bacteroides</i> ;s__uniformis	3.583 ± 0.301 ^c	↑	↑↑	↑	↓↓↓	0.00001
<i>Enterococcus</i> ;s__durans	0.400 ± 0.670 ^b	↑↑↑	↑↑↑↑	↑↑↑	↓↓↓	0.00305
<i>Enterococcus</i> ;s__faecalis	0.005 ± 0.007 ^b	↑↑↑↑	↓	↑↑↑↑	↑	0.00001
<i>Lactobacillus</i> ;s__mucosae	0.003 ± 0.002 ^b	↑↑↑↑	↑↑↑↑	↑↑↑	—	0.0001
<i>Lactobacillus</i> ;s__plantarum	0.001 ± 0.000 ^c	↑↑↑↑	↑↑↑↑	↑↑↑↑	—	0.00001
<i>Faecalibacterium</i> ;s__prausnitzii	1.734 ± 0.770 ^a	↑	↑	↑	↓↓↓	0.01857
<i>Akkermansia</i> ;s__muciniphila	0.903 ± 0.122 ^a	↑	↑	↑	↓↓↓↓	0.00869

The major changes observed with HPBA are the increase in "probiotics" *Bifidobacteriaceae* and *Lactobacillales*, beneficial taxa such as *Faecalibacterium* and *Akkermansia*, and fibrolytic taxa such as *Bacteroides*

MICROBIOMICS: Shifts in the abundance of opportunistic taxa

Miseq

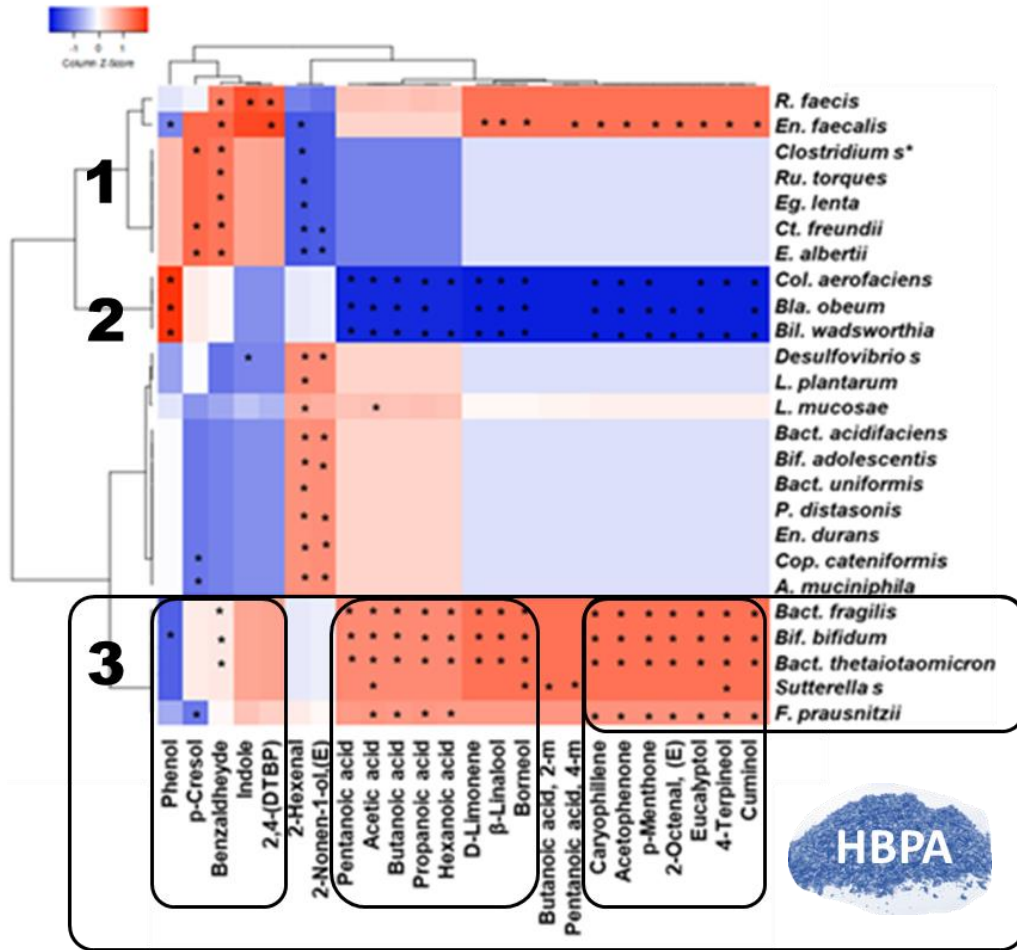
1)
2)
3)
4)

Taxon	% relative abundance	Log ₂ (F/C)				P value
		changes				
	Baseline Mean	HPBA	FOS 24 h	HB 24 h	BC 24 h	
Opportunistic taxa						
<i>Bilophila;s__wadsworthia</i>	0.149 ± 0.019 ^b	↓↓↓	↓↓↓	↓↓↓	↑↑↑	0.00006
<i>Citrobacter;s__freundii</i>	0.051 ± 0.030	↓	↓↓↓↓	↓	↑↑	0.05761
<i>Escherichia;s__albertii</i>	0.064 ± 0.042 ^b	↓	↓	↓	↑↑↑	0.00202
<i>Desulfovibrio;s__</i>	0.395 ± 0.117 ^a	↓↓	↓↓↓	↓↓↓	—	0.04574
<i>Blautia;s__</i>	6.422 ± 1.734	↓↓↓↓	↓↓↓↓	↓↓↓↓	↓	0.05643
<i>Blautia;s__obeum</i>	0.977 ± 0.205	↓↓↓↓	↓↓↓↓	↓↓↓↓	↓	0.02086
<i>Ruminococcus;s__gnavus</i>	2.203 ± 0.720	↓↓↓↓	↓↓↓↓	↓↓↓↓	—	0.04652
<i>Collinsella;s__aerofaciens</i>	2.389 ± 0.747	↓↓	↓↓	↓↓	↑	0.04331
<i>Eggerthella;s__lenta</i>	0.053 ± 0.015	↓↓	↓↓↓	↓↓	↑	0.04445
<i>Sutterella;s__</i>	1.941 ± 0.595	↑↑	—	↑↑	↓	0.01943
<i>Prevotella;s__disiens</i>	0.047 ± 0.080	↓↓↓↓	↓↓↓	↓↓↓	—	0.03669

The major changes observed with HPBA are the reduction of opportunistic and pathobiont taxa of the sulfate-producing *Enterobacteriaceae*, *Clostridiaceae*, and *Collinsella* spp.

INTEROMICS: prebiotic potential

HBPA correlations bacteria/metabolites



In the HBPA dataset, cluster 3 contains the variables describing the prebiotic potential of HPBA: MCFAs, terpenes, *B. bifidum*, *F. prausnitzii* and *Sutterella*

Prebiotic Index

$$qPI = qPCR \text{ Prebiotic Index } \#$$

$$qPI = (Bifidobacteriaceae/Eubacteria) - (Enterobacteriaceae/Eubacteria) + (Lactobacillales/Eubacteria) - (Clostridium \text{ group I}/Eubacteria)$$

Substrate type	qPCR Prebiotic Index (qPI) (24 h)
HBPA	0.866 ± 0.07 ^a
FOS	0.989 ± 0.11 ^a
HB	0.566 ± 0.11 ^b
BC	0.038 ± 0.03 ^c

Normalized values by mean centering method. Scale 0 – 1.

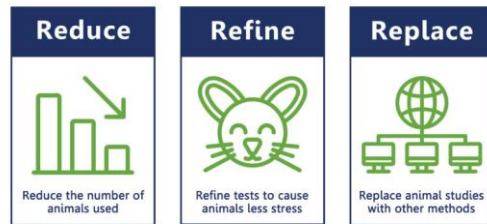
The prebiotic index indicates:

FOS > HBPA > HB

Take home messages

HBPA exerts functionalities towards the gut microbiota of healthy humans

The 3 R's of Animal Research



(EU 2010/63)

- I) More production of SCFAs & MCFAs
- II) Reduction of phenols & indoles
- III) Release of bioactives
- IV) Increased abundance of beneficial taxa
- V) Reduced abundance of opportunistic taxa
- VI) Retention of Eubiosis
- VII) Prebiotic signature



postbiotic HBPA will soon be tested on cell line models

This study structures to the principles of PRECISION NUTRITION and can be re-arranged for studies of PERSONALIZED NUTRITION

This study serves as a pre-clinical basis and can be validated by in vivo trials

Postbiotic = preparation of inanimate microorganisms and/or their components that confers a health benefit on the host

Valorization of byproducts from the plant Queen of Sustainability!

This study save the life of at least 30 mice!



ALMAIDEA



**CENTRO INTERDIPARTIMENTALE
DI RICERCA INDUSTRIALE
AGROALIMENTARE (CIRIAGRO)**



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